

Original Article

Alkaloid Screening and HPTLC Fingerprinting of the Ethanolic Leaf Extract of *Datura innoxia* Mill. with In Silico Evaluation of Scopolamine at the Human M2 Muscarinic Receptor

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ABSTRACT

Objectives: To prepare the ethanolic leaf extract of *Datura innoxia* Mill., screen it for alkaloids, establish its high-performance thin-layer chromatography (HPTLC) fingerprint, and use molecular docking to examine how scopolamine binds the human M2 muscarinic receptor. **Methods:** Dried leaf powder was extracted with ethanol in a Soxhlet apparatus. Alkaloids were screened with Dragendorff's, Mayer's, Wagner's and Hager's reagents. HPTLC was performed on silica gel 60 F254 plates with toluene:ethyl acetate:formic acid (5:4:1, v/v/v) at five extract concentrations (25–150 mg/mL) in two independent runs, with detection at 254 and 366 nm and densitometric scanning. Scopolamine was docked into the M2 receptor orthosteric site (PDB ID: 3UON); the co-crystallised antagonist quinuclidinyl benzilate (QNB) served as a reference. **Results:** All four alkaloid reagents gave positive reactions. HPTLC produced a reproducible banding pattern in both runs, with band intensity rising with extract concentration. Scopolamine docked with a best binding energy of -9.40 kcal/mol (predicted K_i 128.32 nM), compared with -11.86 kcal/mol (2.04 nM) for QNB. The ten poses formed one cluster (cluster RMSD ≤ 0.94 Å), stabilised by a salt bridge with Asp103, an aromatic cage formed by Tyr104, Trp400, Tyr403 and Tyr430, and a hydrogen bond with Asn404. **Conclusion:** The leaf extract contains alkaloids and yields a reproducible HPTLC fingerprint, and scopolamine is predicted to occupy the M2 orthosteric pocket in the canonical antagonist mode. The presence of scopolamine in the extract and any sedative effect need confirmation by co-chromatography or mass spectrometry and by in vivo testing.

Key words: *Datura innoxia*; scopolamine; HPTLC fingerprint; molecular docking; M2 muscarinic.

Medicinal plants continue to be an important source of therapeutically useful chemicals and one of the more significant types of plant constituents includes alkaloids [1]. *Datura innoxia* Mill. (Thorn Apple; Solanaceae family) is a branched medicinal herb of tropical and warm temperate zones that has been traditionally used for asthma, inflammation, wounds, rheumatism and pain relief [2,3]. Its characteristic phytochemicals include the tropane alkaloids hyoscyamine, atropine and scopolamine, whose concentration depends on plant part, development stage, place of growth and method of extraction [2,4,5]. Scopolamine (hyoscine) is a competitive, non-selective antagonist at muscarinic acetylcholine receptors. treatment of motion sickness and vomiting.

It readily passes through the blood brain barrier and is used for Amongst other things, it exerts sedative and memory impairing actions [6,7]. At the same time, these are also the same phytochemicals which make the plant poisonous causing symptoms like dry mouth, dilated pupils, increased heart rate, confusion, delirium and coma in serious cases [2,8]. Cytotoxic and genotoxic activity of the leaf extract and narcotic effect of the seed extract in mice have also been documented, therefore extracts must be chemically characterised before any pharmacological claims are made about them. Determination of scopolamine content in different organs of *D. innoxia* has been done using gas and liquid chromatography [4,5,9,10].

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High performance thin-layer chromatography (HPTLC) provides an economical way to generate the fingerprint of herbal extracts that facilitate its identification and quality control. Molecular docking studies could complement the profiling approach by giving a preliminary structure based information about the potential binding properties of the component. The crystal structure of the human M2 muscarinic receptor in complex with QNB (quiniucidinyl benzilate antagonist) (PDB ID: 3UON) [11] would serve as the suitable template for such docking studies.

MATERIALS AND METHODS

Plant material

Fresh leaves of *D. innoxia* were collected from 11.934655, 79.373712 collection site and 28 March 2026, authenticated by Dr.N Ashok Kumar, M.Sc., Ph.D. botanist. Damaged leaves and foreign matter were removed. The leaves were washed, shade-dried at room temperature for three to four weeks until constant dryness, pulverised in a mechanical grinder, passed through 40 sieve number and stored in an airtight container.

Chemicals and equipment

Ethanol, methanol, toluene, ethyl acetate and formic acid were of analytical grade Deep Molecule Drug Discovery Solutions [Regd. R&D: IN -0126-9385MM]. HPTLC was carried out on silica gel 60 F254 plates with a CAMAG Linomat 5 applicator, a twin-trough chamber and a CAMAG TLC Scanner. The scopolamine reference standard was Deep Molecule Drug Discovery Solutions [Regd. R&D: IN -0126-9385MM].

Extraction

Powdered leaf 20 g was extracted with 100 mL of ethanol in a Soxhlet apparatus (12) for 6 h. The extract was concentrated by [Rotary evaporation], weighed to calculate the yield (% w/w) and stored in a sterile glass container until analysis.

Alkaloid screening

A small amount of the extract was dissolved in dilute hydrochloric acid solution, warmed gently, and filtered. One milliliter aliquots of the filtrate were separately tested for Dragendorff's reagent (precipitate orange or orange red in color), Mayer's reagent (precipitate cream or pale yellow in color), Wagner's reagent (precipitate reddish brown in color) and Hager's reagent (precipitate yellow in color) [13]. Acid without any extract served as blank for all tests. A test was considered positive (+) if the precipitate appeared as expected, negative (–) otherwise.

HPTLC fingerprinting

The extract was prepared in methanol at concentrations of 25, 50, 75, 100, and 150 mg/ml (samples S1-S5). It was sonicated

for 15 minutes and then filtered using a 0.45 µm filter membrane. The plates were activated at 110°C for 20 minutes. The samples were applied as 8 mm bands using 2 µL of the solution. The plates were developed up to a migration of about 80 mm in a twin-trough chamber pre-equilibrated for 20-30 minutes with toluene: ethyl acetate: formic acid (5:4:1 v/v/v), dried, and examined using UV light at 254 and 366 nm. The densitometric scan was done at 254 and 366 nm and the R_f values were noted down. The experiment was conducted independently (n=1 and n=2) on Deep Molecule Drug Discovery Solutions [Regd. R&D: IN -0126-9385MM] from 22 to 24 June 2026.

Molecular docking

The crystal structure of the human M2 receptor with QNB (PDB ID: 3UON) [11] was retrieved from the Protein Data Bank. Water molecules and heteroatoms were removed, polar hydrogens were added and the receptor was converted to PDBQT format. The three-dimensional structure of scopolamine (PubChem CID: 482020663) was energy-minimised, protonated at physiological pH and prepared in PDBQT format with rotatable bonds defined. A grid box (50*50*50, 0.375 Å) was placed on the orthosteric site defined by the position of QNB. Docking was performed with [AutoDock 4.2 with the 10 Runs] [14]. Co-crystallised QNB was re-docked as a reference. Binding energies (kcal/mol) and predicted inhibition constants (K_i) were taken from the docking output and poses were grouped by cluster RMSD. Interactions were examined in [PyMOL].

Data analysis

Observations were descriptive. Qualitative tests were scored as positive or negative, and HPTLC results are reported as band positions (R_f) and agreement between two runs. No inferential statistics were applied, so no significance level was set.

RESULTS

Extract yield

Soxhlet extraction of 10 g of leaf powder gave 9.36 g or 93.6 % w/w of ethanolic extract.

Alkaloid screening

All four reagents gave positive reactions (Table 1; Figure 1). Dragendorff's reagent gave an orange-red precipitate, Mayer's reagent a cream to pale-yellow precipitate, Wagner's reagent a reddish-brown precipitate and Hager's reagent a yellow precipitate, whereas the blanks showed no precipitate. These are general alkaloid tests and cannot separate scopolamine from hyoscyamine or atropine, so chromatographic analysis followed.

Table 1. Alkaloid screening of the ethanolic leaf extract of *D. innoxia*

Test	Reagent	Observation	Result
Dragendorff's	Potassium bismuth iodide	Orange-red precipitate	Positive
Mayer's	Potassium mercuric iodide	Cream/pale-yellow precipitate	Positive
Wagner's	Iodine–potassium iodide	Reddish-brown precipitate	Positive
Hager's	Saturated aqueous picric acid	Yellow precipitate	Positive

Blanks (acid without extract) showed no precipitate.

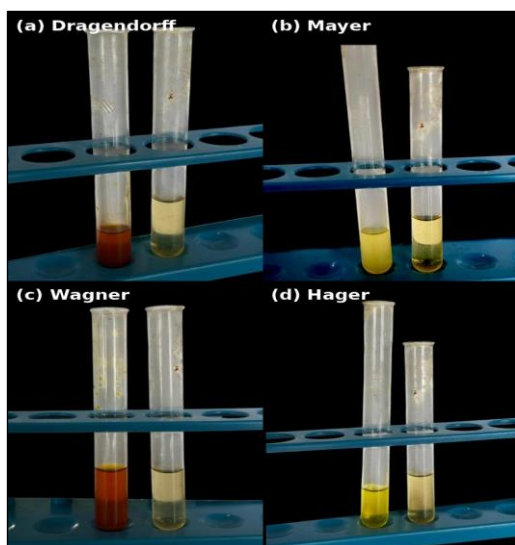


Figure 1. Alkaloid tests on the acidic filtrate of the extract: (a) Dragendorff's, (b) Mayer's, (c) Wagner's and (d) Hager's reagents. In each pair the left tube holds the extract and the right tube the blank.

HPTLC fingerprint

The sample produced several sharp bands in the presence of UV illumination (Figure 2). There were no differences between the banding pattern in the two separate experiments, and there was no difference in band position among all five different concentrations. The band intensity varied gradually from S1 (25 mg/mL) to S5 (150 mg/mL), with S1 and S2 showing weak bands, whereas S3 to S5 showed strong, well-resolved bands. By densitometric scanning, five peaks were detected at R_f = 0.10, 0.22, 0.40, 0.60, and 0.82 (Figure 3; Table 2).

Table 2. R_f values of the peaks resolved by densitometric scanning of the extract

Peak	R_f
1	0.10
2	0.22
3	0.40
4	0.60
5	0.82

R_f , retardation factor. Scanning wavelength: 254 nm and 366 nm.

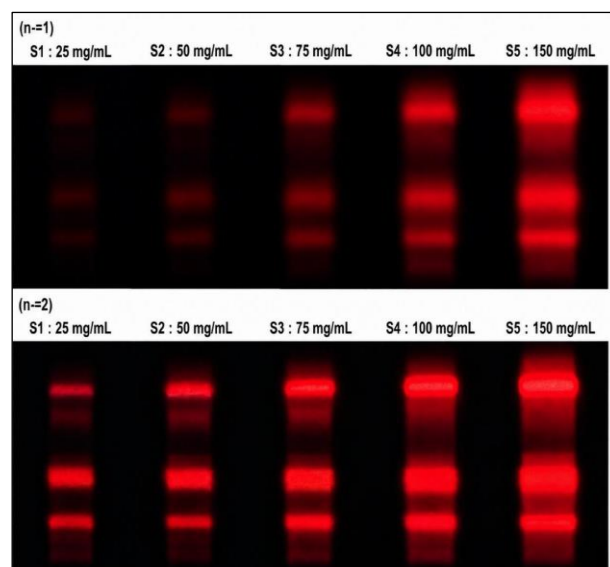


Figure 2. HPTLC fingerprint of the extract at 25–150 mg/mL (S1–S5) under UV light; two independent runs ($n = 1$ upper, $n = 2$ lower).

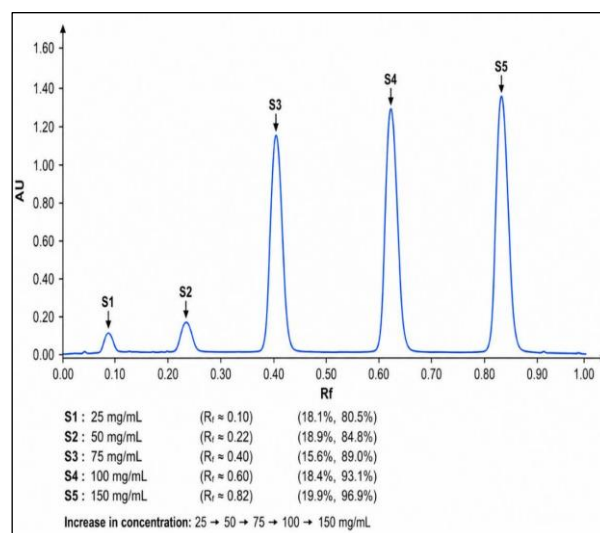


Figure 3. Densitometric chromatogram of the extract showing peaks and their R_f values.

Molecular docking

Scopolamine docked into the orthosteric pocket of the M2 receptor with a best binding energy of -9.40 kcal/mol and a predicted K_i of 128.32 nM. Re-docking of co-crystallised QNB gave -11.86 kcal/mol (K_i 2.04 nM), a difference of 2.46 kcal/mol (Table 3). The ten poses of scopolamine (-8.81 to -9.40 kcal/mol) converged on a single cluster with a cluster

RMSD of 0.94 Å or less (Table 4), indicating a well-defined binding mode. In the best pose (Figure 4), the protonated tropane nitrogen formed a salt bridge with Asp103. The bicyclic ring system lay within an aromatic cage formed by Tyr104, Trp400, Tyr403 and Tyr430, held by π - π and van der Waals contacts. The hydroxyl group of scopolamine formed a hydrogen bond of 2.8 Å with Asn404. The epoxide substituent, which atropine lacks, produced small differences in pose near the rim of extracellular loop 2.

Table 3. Docking scores at the human M2 muscarinic receptor (PDB ID: 3UON)

Ligand	Binding energy (kcal/mol)	Predicted Ki
Scopolamine	-9.40	128.32 nM
QNB (co-crystallised ligand, re-docked)	-11.86	2.04 nM

QNB, quinuclidinyl benzilate; Ki, inhibition constant.

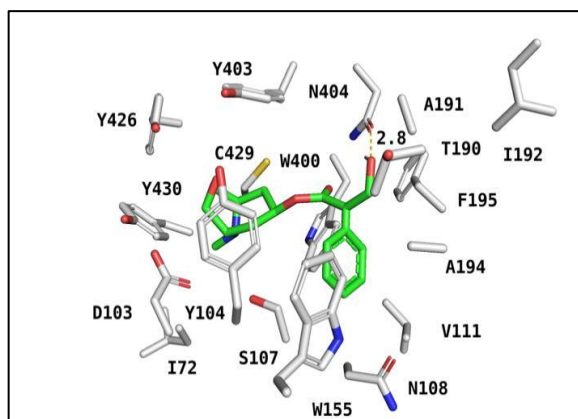


Figure 4. Best-scoring pose of scopolamine (green sticks) in the orthosteric site of the human M2 receptor (PDB ID: 3UON). Residues within the pocket are labelled; the dashed line is the 2.8 Å hydrogen bond to Asn404.

DISCUSSION

All four alkaloid reagents reacted with the ethanolic leaf extract, whereas the acid blank gave no precipitate. This agrees with the tropane alkaloid content reported for the genus [2,3]. However, these reagents detect alkaloids only as a class. [9] Identified 53 alkaloids in the organs of *D. innoxia* by GC/MS [12], and Plank and Wagner reported that hyoscyamine is converted to scopolamine in the aerial parts [5]. Leaf material is therefore likely to contain several related alkaloids that give the same precipitates, so scopolamine must be separated and identified before any claim is made about it.

The HPTLC fingerprint was reproducible. Five concentrations (25–150 mg/mL) gave identical band positions, with intensity rising from S1 to S5, and two independent runs gave the same five peaks (Rf 0.10, 0.22, 0.40, 0.60 and 0.82). This supports its use as a qualitative fingerprint of this extract

under the stated conditions. It is not evidence for scopolamine, for two reasons. The bands were seen under UV at 254 and 366 nm, where scopolamine absorbs weakly, so the fluorescent zones may largely represent non-alkaloidal constituents such as chlorophyll derivatives.

Table 4. Binding energies and RMSD values of the ten scopolamine poses

Pose	Binding energy (kcal/mol)	Cluster RMSD (Å)	Reference RMSD (Å)
1	-9.35	0.41	4.02
2	-9.37	0.41	3.97
3	-8.81	0.94	4.01
4	-9.30	0.38	3.98
5	-9.39	0.28	4.01
6	-9.28	0.63	3.86
7	-8.88	0.90	3.96
8	-9.33	0.28	4.05
9	-9.34	0.36	3.97
10	-9.40	0.00	4.01

Note: RMSD = root-mean-square deviation. Poses are numbered as in the docking output.

Identification by Rf alone is also only presumptive, and co-chromatography with a standard plus GC-MS or LC-MS/MS confirmation, as used in earlier work [4,5,12,13], would be needed. Scopolamine content varies with organ and extraction. Mardare et al. found the lowest ethanolic content in leaves and higher recovery with 1-butanol [4], so our ethanolic leaf extract falls in the lower-yielding combination and its bands should not be assumed to be dominated by scopolamine [2,4,12]. Docking compared scopolamine with the co-crystallised antagonist QNB at the human M2 receptor [14]. QNB scored -11.86 kcal/mol (predicted Ki 2.04 nM) and scopolamine -9.40 kcal/mol (128.32 nM), a difference of 2.46 kcal/mol.

Scopolamine occupied the same orthosteric site, and all ten poses converged on one cluster (cluster RMSD $\leq$ 0.94 Å). A salt bridge to Asp103 and enclosure by aromatic residues are features shared by muscarinic antagonists. The weaker score may reflect the larger hydrophobic surface of QNB packing against the aromatic cage. Docking scores are approximate, however, and predicted Ki values often differ widely from measured affinities, so they are best used to compare ligands within this study and not as estimates of potency. The model also used one receptor subtype, a rigid receptor and no explicit water. Binding to M2 supports antimuscarinic activity but does not demonstrate sedation, which depends on brain penetration, dose and several receptor subtypes [6,7] and can only be tested in vivo.

In summary, the HPTLC findings demonstrate reproducibility of the chromatographic profile but do not, by themselves, establish the identity of scopolamine in the extract. Similarly, molecular docking suggests a plausible binding mode of scopolamine with the M2 receptor; however, docking alone cannot confirm the presence of scopolamine in *D. innoxia* extract. Therefore, further experimental validation is required. The next stage of the study should involve identification and quantification of scopolamine, initially by co-chromatography with an authentic reference standard and subsequently by LC–MS/MS for definitive confirmation and quantitative analysis. Following chemical confirmation, molecular dynamics simulation of the scopolamine–M2 receptor complex may be performed to evaluate the stability of the predicted binding interaction. Finally, considering the known toxicity of *D. innoxia* [2,9], appropriate dose-finding and toxicity studies should be conducted before proceeding to any pharmacological evaluation. This sequential approach will provide stronger chemical, computational, and biological evidence for the proposed activity of the plant extract.

CONCLUSIONS

The ethanolic leaf extract of *D. innoxia* is alkaloid-positive and gives a reproducible HPTLC fingerprint that can serve as a starting point for standardisation once the scopolamine zone is confirmed against a reference standard. Docking indicates that scopolamine can occupy the M2 orthosteric pocket in the canonical antagonist mode, anchored by Asp103 and an aromatic cage, which agrees with its known antimuscarinic action. These findings are preliminary. They justify quantitative analysis of the extract, dynamics simulation of the complex and carefully controlled in vivo assessment of sedative activity, but they do not establish that the extract is sedative.

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